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Sugar changes in young seedlings and decotylized embryos of *Lupinus luteus*

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Abstract:

Mobilisation of sugars in *Lupinus luteus* during the germination and in the early developmental period has been investigated. The embrional decotylized axes of lupin, cultured *in vitro* on mineral medium with sucrose, accumulate an excessive amount of sugars. The reason seems to be the static character of the medium as compared with the metabolism of the cotyledons.

INTRODUCTION

In comparative studies on the metabolism of isolated, decotylized embryos of lupin cultured in vitro and on the axes of normal seedlings, it was possible to establish in the embryos a change in nitrogen turn — over in the direction of an increased protein synthesis (C z o s n o w s k i 1962).

On the basis of further studies (C z o s n o w s k i, M i c h e j d a 1964) a conclusion was drawn that the reason for this phenomenon is among others a rich supply of assimilable sugars from the medium. The static character of the medium was underlined in comparison with the dynamic system in the cotyledons.

In order to establish the effect of sugars from the medium more precisely, an analysis was made of the soluble sugars for comparison of the dynamic changes in the quantitative and qualitative sugar composition in:

a) axes of decotylized embryos, cultured on media with a known concentration of sugar (in this case sucrose), and in

b) axes and cotyledons of seedlings that have been cultured on an inorganic medium without sugar (in this case the cotyledons were the source of sugar and not the medium).

MATERIAL AND METHODS

1. Material and the cultures

Seeds of *Lupinus luteus* cult. Express have been obtained from the Poznań Plant Breeding Station in Kosieczyn. They were collected in 1966 as class S that is elite. The seeds were selected according to size by sieving them. For the experiments seeds with a diameter of 6—7 mm have been chosen. The selected seeds have been dipped in 95% ethanol for one minute, and then they were sterilized for 10 minutes in 0.2% $HgCl_2$. After washing the seeds 5 times in sterile water the seeds were placed in sterile Petri dishes lined with moist filter paper. Dishes with the seeds have been placed into thermostats (at 24° C in darkness). After 24 hours the embryos were isolated aseptically (the embryonal axes were deprived of the cotyledons) and placed on Heller medium with 3% of sucrose and 0.9% agar.

Culturing of the embryos was conducted in two variants, in the dark and under continuous luminescent light of 900 lux.

All the cultures were maintained at a constant temperature of 24° C.

2. Collection and conservation of material

For the analyses we used dry seeds, seeds after 12 and 24 hours of imbibition and embryos or seedlings 2, 3, 5, 7, 9 and 12 days old.

In each case a sample of 100 seeds, embryos or seedlings was used. From the seeds the seed covers, cotyledons and embryos were isolated. Embryos were divided into roots, epicotyls and hypocotyls and the seedlings into roots, hypocotyls, cotyledons and epicotyls. Immediately after separation individual organs were placed into separate flasks containing boiling 95% ethanol with 0.5% $CaCO_3$ added. The boiling was maintained for 10 minutes after placing in ethanol of the last portion of the material. Then the flasks were closed and the material stored in this way until the analyses were made.

3. The analyses

Extraction, removal of fats, removal of proteins and the quantitative determination of carbohydrates was conducted by the method suggested by Biełozierski and Proskuriakow (1954). For the qualitative determinations the extract was further purified by desalting on Dowex IX2-400 in the acetate form. The qualitative analysis was conducted using one directional descending chromatography on Whatman nr. 3 filter paper. The chromatograms were run in n-butanol: acetic acid: water (4 : 4 : 1 v/v) and developed in an alcoholic solution of p-aminophenol acidified with orthophosphoric acid and dried at 80° C for 10 minutes. The sugars were identified by comparison with standard substances run parallely.

RESULTS

1. Qualitative analysis

The chromatograms presented (Figs. 1, 2 and 3) represent the content of individual sugars in the analysed material. The first column on the left hand side of the chromatograms (designated by the symbol St-standard) represents the distribution of pure sugars taken as standards for comparison with the sugars obtained from the analysed material (following columns). The figures at the base of each column determine the age (in days) of the analysed organs, counting always from the onset of culturing (the moment of placing seeds for imbibition).

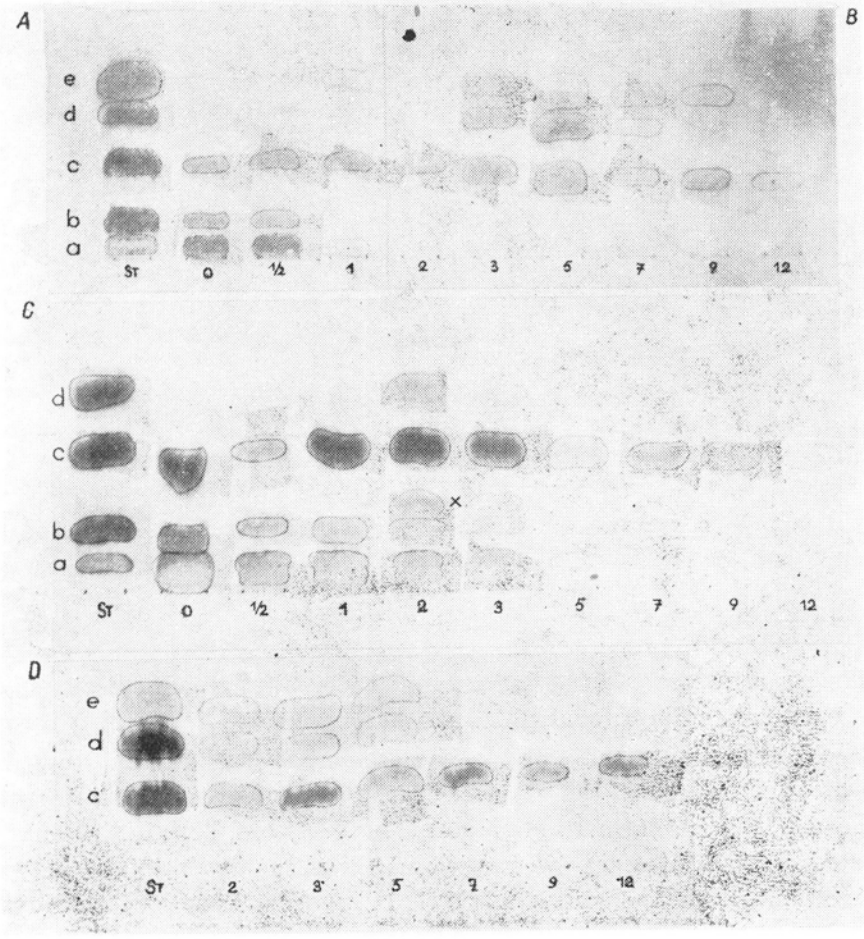

Fig. 1. Saccharides of lupine seedlings

A — Embryos before implantation; *B* — Hypocotyls of seedlings; *C* — Cotyledons of seedlings; *D* — Roots of seedlings

Standard (St): — *a* — stachyose, *b* — raffinose, *c* — sucrose, *d* — glucose, *e* — fructose, *x* — unidentified compound

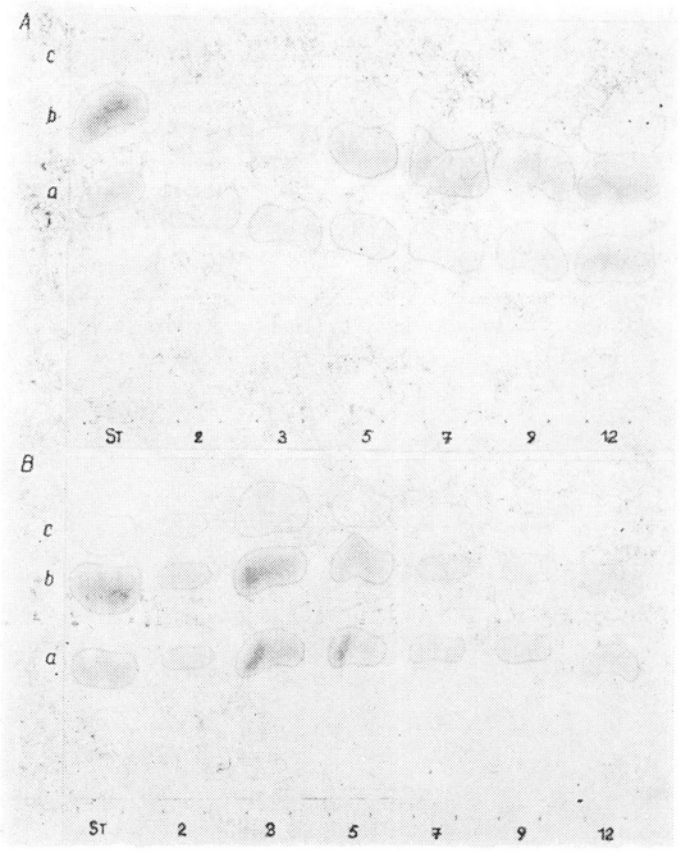

Fig. 2. Saccharides of lupine embryos

A — Hypocotyls of embryos cultured in light; *B* — Roots of embryos cultured in light

Standard (St): *a* — sucrose, *b* — glucose, *c* — fructose

The chromatogram in fig. 1 represents the qualitative changes in sugars from parts of seedlings (control).

In all the organs of seeds and seedlings sucrose can be found (row „C"), regardless of the age of the culture. Stachyose and raffinose is present in dry seeds („O") and in imbibing ones 1/2 or 1 day old, both in the embryos and in the cotyledons. In the cotyledons these sugars disappear between the 3rd and 5th day of culturing, while in seedling axes they are already absent after 24 hours. Glucose and fructose which are absent in the seeds appear after 1 day, primarily in the roots (on the 2nd day) and in the hypocotyl (on the 3rd day). In the root they disappear after 5 and in the hypocotyl after 7—9 days of culturing. In the embryos cultured in vitro (Figs 2 and 3) sucrose is also present all the time. Besides also glucose

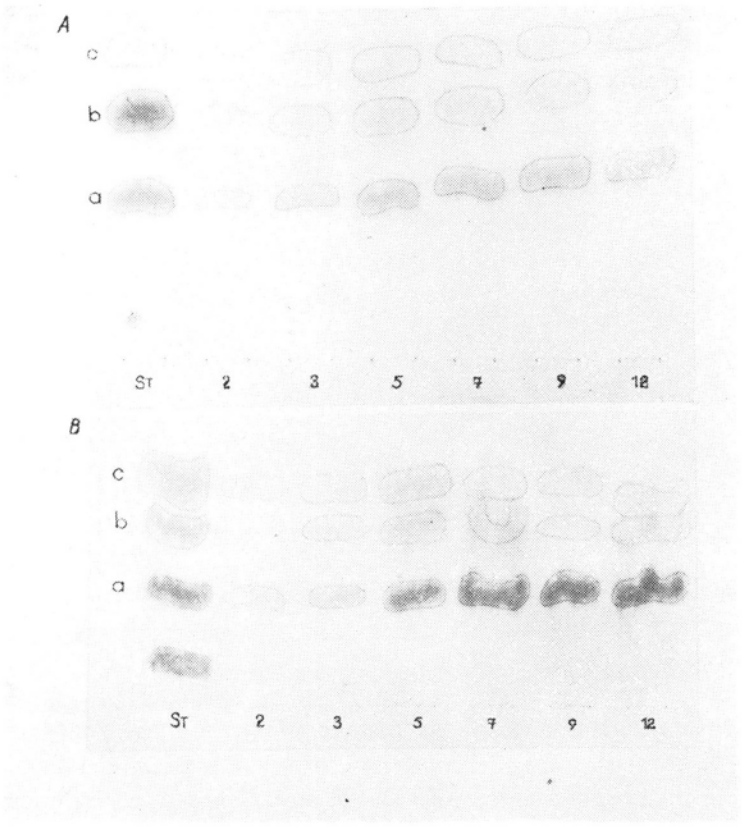

Fig. 3. Saccharides of lupine embryos

A — Hypocotyls of embryos cultured in the dark; *B* — Roots of embryos cultured in the dark

Standard (St): *a* — sucrose, *b* — glucose, *c* — fructose

and fructose are present in large quantities throughout the culture period, to the very end of the experiment, as can be seen even on the qualitative chromatograms.

2. Quantitative anaylsis

Cotyledons of dry, imbibing and germinating seeds contain large quantities of non-reducing sugars (Fig. 4). After two days of culturing the level of sugars in the cotyledons of seedlings rapidly declines.

The axial organs of lupine (Fig. 5*a* and *b*) accumulate sugars till the 5th day of culturing. In older plants the quantity of sugars in the axes also declines. In embryos cultured in vitro (Fig. 5 *c*—*f*) after an initial drop (following placing onto the medium) the quantity of the sugars increases.

In particular the hypocotyls accumulate large quantities of sugars, primarily the reducing ones. In roots the decline in the content of reducing sugars does not take place.

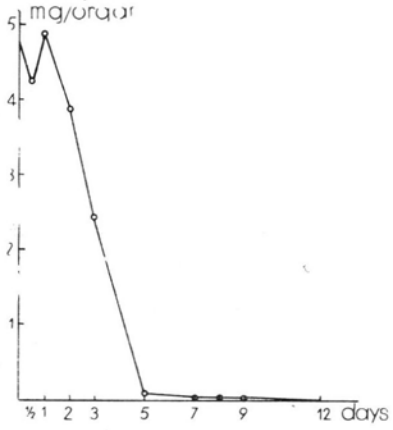

Fig. 4. Content of non-reducing sugars in the cotyledons of lupine

DISCUSSION

The chromatographic analysis has demonstrated the presence of the following sugars: sucrose, glucose, fructose, raffinose and stachyose. The central role in the metabolism of soluble sugars is played by sucrose. This sugar is the main form in which carbohydrates are transported in higher plants (A r n o l d. 1968). In seedlings and embryos of lupine it occurs all the time, in all organs and at all times during culturing.

Glucose and fructose appear in the hypocotyls and roots of seedlings during the third day of germination and disappear in the seventh day. On the other hand in embryos cultured *in vitro,* these sugars occur in large numbers throughout the culture period.

Raffinose and stachyose occur in cotyledons and in seedling axes in the first days of germination.

The results obtained from the qualitative analysis of lupine seedlings agree with the data known from literature concerning the occurence of individual sugars in *Leguminosae* during the germination process and in the early developmental period (W a n n er 1958).

The accumulation of large quantities of glucose and fructose in embryos cultured *in vitro* will be discussed on the basis of the quantitative data.

Axis, and in particular the shoot of a normal seedling accumulates sugars up to the 5th day of germination, after which their quantity quickly declines, (Fig. 5*a, b*) while in embryos after an initial decline (adaptation period after placing onto the medium) a strong absorbtion of sugars from

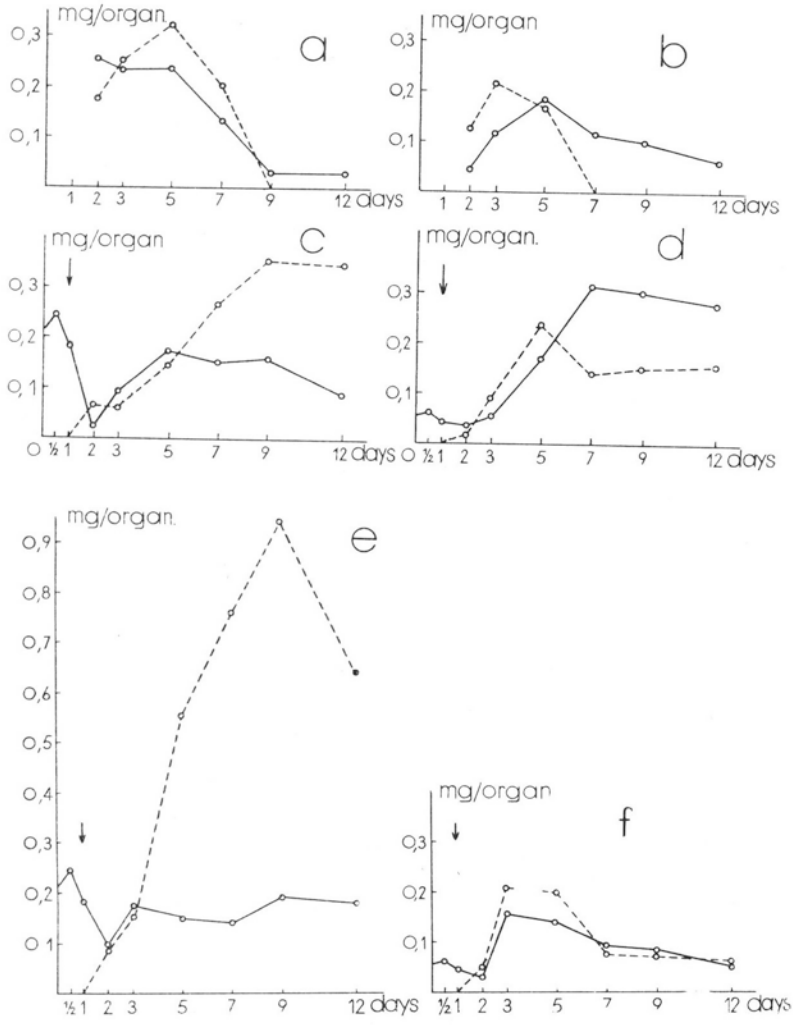

Fig. 5. Content of sugars in the axial organs of lupine

a — hypocotyls + epicotyls of seedlings; *b* — seedling roots; *c* — hypocotyls + epicotyls of embryos cultured in light; *d* — roots of embryos cultured in light; *e* — hypocotyls of embryos cultured in the dark; *f* — roots of embryos cultured in the dark

Continuous line — non-reducing sugars (sucrose); Broken line — reducing sugars (glucose and fructose)

The arrow indicates the time of implantation of the embryos onto the medium

the medium follows and an accumulation greater than in normal seedlings results (Fig. 5c, d e, f). This accumulation was maintained throughout the culture period. It is likely that such a high concentration of sugars in the tissues of the embryo determines the shift of nitrogen pattern towards protein synthesis (C z o s n o w s k i 1962).

Reasons for this excessive accumulation of sugars can be sought by comparing cotyledons of normally growing seedlings with the medium that is supposed to substitute for the cotyledons of which the isolated embryos are deprived.

From the data presented in Fig. 4 it can be calculated, that the concentration of sugars, primarily sucrose, in the water present in the cotyledons of 2 day old seedlings amounts to about 2,6%, in the cotyledons of 3 day seedlings amounts to about 1%, while in cotyledons of 5 day old seedlings already only 0.023%. In the cotyledons of older seedlings the concentration of sugars is even lower. On the other hand the concentration of sucrose in the medium on which the embryos were cultured was 3% and practically did not change throughout the culture period. Thus the medium supplies sugars for the isolated embryos differently than the cotyledons. This confirms the conclusion that the static medium does not substitute for the dynamic systems of the cotyledons (Czosnowski, Michejda 1964). This character of the medium is reflected in the whole metabolism of the isolated embryos and it is difficult to determine all the factors responsible for the different reactions of the embryos compared with normal seedling axes. One of the reasons will no doubt lie in the osmotic conditions. Accumulation of sugars in the embryos in the latter period of their culture could have been caused by the need to maintain turgor during growth on a relatively osmotically concentrated medium. This is indicated by the accumulation of glucose and fructose (particularily in the shoot which is more sensitive to water stress), which give a higher osmotic concentration than sucrose, from the breakdown of which they undoubtedly originate, (Figs 5 c and e). A further argument to support this view can be found in the fact that there is a much greater accumulation of sugars in the shoots of embryos cultured in the dark (etiolated), which had a higher fresh weight. In the results we have not presented the measurements of fresh and dry weight because they concurred with data reported earlier (Czosnowski 1962).

We shall only present as an example the data concerning the shoots of 9 day old embryos, in which we find the maximum of abnormal sugar accumulation.

Organ	Water content (fresh-dry wt.	Molar conc. of sucrose	Molar conc. of glucose and fructose	Osmotic concentr.
Shoot of a 9 day old embryo in light	41 mg	0.012 M	0.048 M	0.06 M
Shoot of a 9 day old embryo in the dark	91 mg	0.006 M	0.057 M	0.063 M

The agreement in the osmotic concentration of the sugars obtained under light and in the darkness (0.06 M and 0.063 M) confirms the role of the osmotic factor in the accumulation of sugars.

The medium contained sucrose with a molar concentration of about 0.09 M and this concentration has resulted in an excessively high accumulation of sugars in the embryos.

How then can we explain the reported need for the use of high concentrations of sucrose in a medium in order to enable the embryo to develop (H o f f m a n n o w a 1964). An answer can be found in the results obtained from the analyses of normal seedlings. The seedling axis in the early period of its development (to the 7th day of germination) accumulates considerable quantities of sugars necessary in this phase (Fig. 5*a*, *b*). Their appropriate supply is regulated by the cotyledons (Fig. 4), which supply initially considerable quantities of sugars, however from the 5th day onwards the supply of sugars rapidly declines as a result of which after 7 days their concentration in the axes is reduced. Thus the embryos have to be implanted onto a medium with high sugar concentration in order to develop during the early phases, whereas in a later phase this concentration becomes too high. R i e t s e m a and others (1953) have been able to show that the optimal concentration of sucrose in the medium for the embryos of *Datura stramonium* cultured in vitro fluctuates depending on the developmental phase (immature embryos) during which the embryos have been isolated. The youngest, immature embryos required 8% of sucrose while for the mature ones 0.5% was sufficient.

On the basis of the above discussion and by analogy to the cited reports we wish to suggest that lupine embryos should be placed first on a medium rich in sugars and in a later stage of their culture on a medium with lower sugar concentrations. In this way the medium can become more dynamic, in that besides sugars the levels of other metabolites can also be varied in supply depending on the needs of axial organs.

SUMMARY

A qualitative and quantitative analysis was made for sugars in embryos (decotylized embryonic axes) of lupin cultured *in vitro* on a Heller medium with 3% sucrose and in seedlings with the cotyledons intact, cultured parallel on a medium without sucrose.

1. In the embryos cultured *in vitro* a considerable accumulation of sugars was observed, particularily of glucose and fructose.

2. In the axial organs of control plants glucose and fructose accumulate only between the third and seventh day of culture and to a lesser degree.

3. Cotyledons of control plants supply sugars in a dynamic way to the axial organs. During imbibition and early germination phase they supply large amounts of sugars but in later phases much less.

The differences were underlined between the cotyledons and the medium which was supposed to substitute for them.

A static medium supplies to the embryos large quantities of sugar throughout the culture period, which results in a shifting of the nitrogen metabolism in the direction of protein synthesis.

The role of osmotic factor in the medium as well as the reasons for the excessive accumulation of sugars in the embryos was discussed.

REFERENCES

Arnold W. N., 1968, The selection of sucrose as the translocate of higher plants., J. Theoret. Biol. 21: 13—20.

Biełozjerski A., Proskuriakow N., 1954, Ćwiczenia z biochemii roślin, Warszawa — PWRiL.

Czosnowski J., 1962, Metabolism of excised embryos., Acta Soc. Bot. Pol. 31: 693—702.

Czosnowski J., Michejda J., 1964, Metabolism of excised embryos of *Lupinus luteus* L. IV. *In vitro* growth as compared with in vitro growth of germ axes decotylized at different phases of germination., Acta Soc. Bot. Pol. 33: 335—350.

Hoffmannowa A., 1964, Some aspects of sugar nutrition of excised embryos of Lupinus luteus L. and Brassica oleracea L., Acta Soc. Bot. Pol. 1: 193—210.

Rietsema J., Satina S., Blakeslee A. F., 1953, The effect of sucrose on the growth of Datura stramonium embryos in vitro., Am. J. Bot. 40: 538—545.

Wanner H., 1958, Mobilisierung der Kohlenhydrate bei der Keimung., Handbuch der Pflanzenphysiologie, W. Ruhland, Band 6: 935—940.

Cukry rozpuszczalne w siewkach i hodowanych bezliścieniowych zarodkach Lupinus luteus

Streszczenie

Przeprowadzono badania jakościowe i ilościowe cukrów rozpuszczalnych w zarodkach (embrionalnych osiach siewek pozbawionych liścieni) łubinu *Lupinus luteus* cv. Express, hodowanych na pożywce z 3% sacharozy przez 12 dni na świetle i w ciemności.

Kontrolnie przebadano cukry w poszczególnych organach roślin nie pozbawionych liścieni, hodowanych równolegle na pożywce mineralnej.

W zarodkach hodowanych *in vitro* wykryto duże nagromadzanie cukrów zwłaszcza glukozy i fruktozy, utrzymujące się do końca hodowli.

W organach osiowych roślin kontrolnych cukry te występują tylko okresowo, między trzecim a siódmym dniem hodowli, a następnie zanikają. Stwierdzona duża ilość cukrów w hodowanych zarodkach może służyć jako wyjaśnienie przegięcia w ich gospodarce azotowej w kierunku wzmożonej syntezy białek.

Nadmierne nagromadzanie cukrów w zarodkach wyjaśniono przez porównanie zmian stężeń cukrów w liścieniach roślin kontrolnych z zawartością cukrów w pożywce, która miała zastąpić liścienie zarodkom. Liścienie dynamicznie regulują dostawę cukrów do organów osiowych. Podczas pęcznienia i w pierwszych dniach kieł-

kowania dostarczają do osi duże ilości cukrów w stężeniu podobnym do stosowanego w pożywce. W późniejszym okresie dostarczają cukrów bardzo mało. Statyczna pożywka zawierała przez cały czas hodowli to samo wysokie stężenie cukru.

Przedyskutowano rolę czynnika osmotycznego. Nagromadzanie cukrów przez zarodki może być reakcją na siłę ssącą pożywki.

W konkluzji zaproponowano udynamicznienie hodowli *in vitro* przez przenoszenie zarodków podczas hodowli na nową pożywkę, naśladującą liścienie w aktualnej fazie rozwoju.